Application Note:
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Large Scale Recombinant Protein Production Using Thermo Scientific Sorvall Superspeed Centrifuges and Carbon Fiber Rotors

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KEY WORDS

- Recombinant Protein Production
- Proteomics
- Large Volume Pelleting
- Fermentors
- Transformation
- Superspeed Centrifuges
- Carbon Fiber Rotors

Introduction

Large scale recombinant protein production is becoming increasingly important for applications in the field of proteomics. Protein characterization and functional studies can provide clues about the mode of action and can facilitate development of therapeutic drugs. For instance, using structural biology, inhibitors can be made that bind to, and inactivate, disease-related enzymes. Additionally, analysis of protein-DNA interactions enables scientists to better understand the expression of disease-related genes. In other cases, protein structure has aided in the study of antigen-antibody interaction and ligand-receptor binding, helping to unlock mysteries in immunology and molecular signal transduction.

However, to successfully study protein structure and function, large quantities of protein (mg to g amounts) must be isolated from an appropriate expression system, such as a bacterial (e.g. *Escherichia coli*) or eukaryotic cell-based expression system (e.g. yeast, insect, mammalian). In order to meet this need, cell cultures can be grown in large volume in a shake flask culture or by the implementation of fermentation techniques. Using high cell density or fermentation, a researcher can optimize cell culture conditions to increase cell mass and recombinant protein yield.

This application brief describes the usage of Thermo Scientific Sorvall Superspeed centrifuges and large volume rotors to increase the efficiency of cell harvesting prior to protein isolation.

Thermo Scientific Sorvall RC6 Plus and Evolution RC Superspeed Centrifuges with Thermo Scientific FIBERLite® Carbon Fiber Rotors

Materials and Methods

During high volume cell culture, translated recombinant protein is secreted into the culture medium and/or remains intracellular. In either case, large scale centrifugation marks the first step in preparing the recombinant protein. Listed below are basic protocols for protein production in large volume bacterial cell cultures (up to 4 L).

Procedures

PROTOCOL 1: Basic Protocol for the Production of Recombinant Protein in Bacterial Cells *(Protocol Adapted from Current Protocols in Protein Science, Bernard and Payton, 1995-1)*

1. Inoculate 100 mL flask containing 20 mL LB medium with *Escherichia coli (E. coli)* expressing the gene of interest. Shake overnight at 37°C. If needed, one protocol for the transformation of *E. coli* with a gene of interest is detailed in protocol 3. Other methods for the induction of gene expression in bacterial cells can be conducted using many commercially available transfection kits.
2. Transfer overnight culture to 800 mL LB medium in a 3-L Erlenmeyer flask. Incubate and shake cells 1 to 2 hr at 37°C.
3. After cells reach exponential growth phase (OD_{600} of 0.5 to 0.6), transfer cells to a 500 mL – 1 L chilled centrifuge bottle.
4. Place centrifuge bottles in the F9S-4x1000y, F12S-6x500y, or the SLA-3000 rotors (depending on volume) and centrifuge in the Thermo Scientific Sorvall Evolution RC or Thermo Scientific Sorvall RC6 Plus for 15 min. at 4000 x g, 4°C.
5. Pellet may then be resuspended in various medium(s) for subsequent protein analysis.

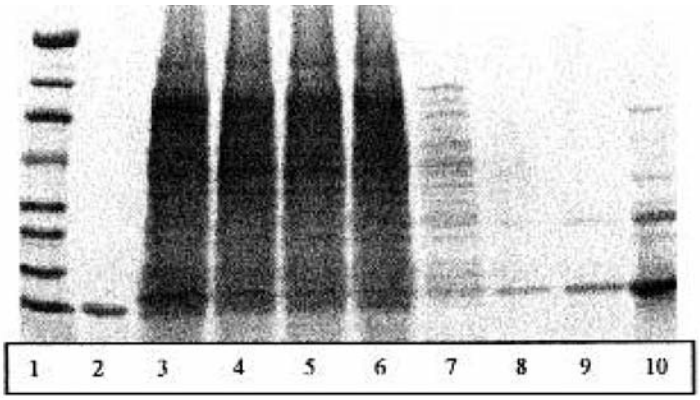

Figure 1. 4-20% SDS-PAGE of IL-8 NiNTA Purification. 20 mg of IL-8 was produced in a 4 L fermentation run, which is a ten-fold increase in production over a previous shake flask culture of the same total volume.

1. Bio-Rad® Prestained MW Marker
2. IL8 Standard (2 µg)
3. NiNTA Load
4. NiNTA Flow Through 1
5. NiNTA Flow Through 2
6. NiNTA Flow Through 3
7. NiNTA Wash 1
8. NiNTA Wash 2
9. NiNTA Eluate 1
10. NiNTA Eluate 2

PROTOCOL 2: Utilization of Fermentation to Enhance Recombinant Protein Production
(Protocol Adapted from Current Protocols in Protein Science, Bernard and Payton, 1995-2)
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In a recent recombinant human Interleukin-8 production run, our group achieved a two-fold increase in the cell density of a 4 L batch culture as compared to a previous shake flask experiment, and a ten-fold increase in rhIL-8 yield (Figure 1).

The fermentation timeline followed that of the shake flask experiment, and no nutrient additions were made to the media. The only differences in the experiments were that the dissolved oxygen was controlled at 30% (vs. uncontrolled in the shake flask), and the temperature was maintained at 32°C instead of 37°C during the cell growth phase. To optimize culture conditions in future experiments, pH will be regulated and glucose will be added to the media. Optimizing these two parameters alone will increase cell density at least six-fold (unpublished observations), and we expect a concomitant increase in recombinant protein yield.

A basic protocol for utilization of fermentation to produce results similar to those above is as follows (please follow manufacturer's instructions for fermentation use):

1. Prepare the inocolum and fermentor.
   a. Prepare an overnight culture of transformed *E. coli* strain expressing the protein of interest.
   b. Grow culture until stationary phase is reached (OD_{600} >1.5). Use overnight culture to inoculate the fermentor (volume is 1-10% of intended fermentation volume).
2. Sterilize the fermentor and medium.
3. Inoculate the fermentor, growth cells, and induce protein expression.
   a. Inoculate fermentor by transferring inoculum aseptically into reactor vessel.
   b. Monitor growth by measuring OD_{600} of samples at 1 hr. intervals. When target cell concentration is reached, proceed to induction. Target cell concentration may be within an OD_{600} of 5 to 20.
   c. Induce protein expression via temperature or chemical induction.
4. Harvest the cells.
   a. For small fermentors (1-10 L)
      i. Pressurize the fermentor and transfer the reactor contents into an intermediate container.
      ii. Aliquot culture into 500 mL or 1 L centrifuge bottles.
      iii. Centrifuge at 5000 x g for 15 min., 4°C in the F9S-4x1000y, F12S-6x500y, or the SLA-3000 rotors (depending on volume) and Evolution RC or RC6 Plus.
5. Store the harvested cells and clean the fermentor.
   a. Collect pellets from each centrifuge bottle and place together in a heat-sealed plastic bag. Pellets may be frozen at -20°C and use for subsequent protein analysis.

PROTOCOL 3: Production of Recombinant Protein by Electroporation of *E.coli* cells
(Adapted from Current Protocols in Protein Science, Bernard and Payton, 1995-1)

1. Prepare competent cells
   a. Inoculate 100 mL flask containing 20 mL LB medium with a single colony of the *E. coli* host strain to be transformed. Shake overnight at 37°C.
   b. Transfer overnight culture to 800 mL LB medium in a 3-L Erlenmeyer flask. Incubate and shake cells 1 to 2 hr. at 37°C.
   c. After cells reach exponential growth phase (OD_{600} of 0.5 to 0.6), transfer cells to a 500 mL to 1 L chilled centrifuge bottle.
   d. Place centrifuge bottles in the F9S-4x1000y, F12S-6x500y, or the SLA-3000 rotors (depending on volume) and centrifuge in the Evolution RC or RC6 Plus for 15 min. at 4000 x g, 4°C.
   e. Discard supernatant and resuspend pellet in 5-10 mL of ice cold water. Bring to a final volume of 800 mL with ice-cold water. Repeat twice.

f. Resuspend pellet in ice-cold water to a final volume of 50 mL. Pellet again in a chilled 50 mL centrifuge tube.

g. Discard supernatant, estimate the volume of the pellet, and add an equal volume of ice-cold water.

h. Vortex, distribute 100 µL aliquots into prechilled 1.5 mL sterile microcentrifuge tubes. Cell aliquots may be used immediately or stored in a -80°C freezer.

2. Transform competent cells

a. Add 5 pg to 0.5 µg plasmid DNA to 100 µL of competent cells. Mix by inverting tubes several times.

b. Set electroporation apparatus to 2.5 kV, 25 µF, 200 Ω.

c. Transfer DNA and cells to prechilled electroporation cuvette, place cuvette into electroporation chamber and apply the electrical pulse.

d. Remove cuvette, add 1 mL SOC medium and transfer contents to a 20 mL sterile culture tube. Incubate 60 min. with moderate shaking.

e. Plate transformation culture (10 to 100 µL) onto LB plates to isolate single colonies. Single colonies can then be picked to produce pure and viable cultures of the bacteria expressing your protein of interest.

Conclusion

In order to produce large quantities of protein for proteomics work, cell cultures must be large volume or cell dense. Fermentation improves efficiency by increasing cell density and provides easier handling of large cultures (>4 L), since the culture is contained in one vessel as compared to handling several shake flasks. Pelleting of bacterial cells from a large volume culture can be completed in the F9S-4x1000y, F12S-6x500y, or SLA-3000 rotors in Superspeed centrifuges. The carbon fiber rotors (F9S-4x1000y and F12S-6x500y) are lightweight, and thus easy to handle, and can process 3-4 L of cell culture in a single run. The time saved in centrifugation with these large volume rotors improves the efficiency of downstream processing of recombinant proteins.

References

1. Bernard, Alain and Mark Payton. Selection of *Escherichia coli* Expression Systems. *Current Protocols in Protein Science* 5.2.1-5.2.18. (1995-1)

2. Bernard, Alain and Mark Payton. Fermentation and Growth of *Escherichia coli* for Optimal Protein Production. *Current Protocols in Protein Science* 5.3.1-5.3.18. (1995-2)

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